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Comparison of Cytotoxic T-Lymphocyte Responses to Hepatitis C Virus Core Protein in Uninfected and Infected Individuals

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Cytotoxic T lymphocytes have been implicated in the control of hepatitis C virus (HCV) infection. Recognition by cytotoxic T lymphocytes of epitopes within HCV core protein has been defined previously by in vitro stimulation with synthetic peptides. The aim of this study has been to examine cytotoxic T-lymphocyte responses generated against peptides produced naturally following intracellular processing of viral protein. Antigen-specific cytotoxic T-lymphocyte lines were generated from both HCV uninfected and infected individuals by culturing $CD8^+$ T cells with autologous dendritic cells loaded intracytoplasmically with recombinant HCV core protein. Analysis of the epitopes recognized by core protein-specific cytotoxic T lymphocytes used synthetic peptides that were selected based on their predicted binding to HLA-A*0201 molecules. Core protein-specific cytotoxic T lymphocytes derived from HCV uninfected and infected individuals were able to lyse autologous target cells pulsed with each of 5 predicted epitopes. Generation of HCV-specific cytotoxic T lymphocytes using dendritic cells as antigen presenting cells provides a method of comparing the potential repertoire of cytotoxic T-lymphocyte responses to the responses that occur in chronically infected individuals. No evidence of a qualitatively different response by patient cytotoxic T lymphocytes was apparent which might explain persistence of the virus. ***J. Med. Virol. 58:239–246, 1999.*** © 1999 Wiley-Liss, Inc.

KEY WORDS: dendritic cells; HLA-A*0201; synthetic peptides

INTRODUCTION

Hepatitis C virus (HCV) is the predominant cause of transfusion-associated and sporadic non-A, non-B hepatitis [Choo et al., 1989]. The spectrum of liver disease associated with HCV varies from mild chronic hepatitis to cirrhosis and ultimately to hepatocellular carcinoma [Tong et al., 1995]. Chronic liver disease is associated with persistence of the virus, although the mechanisms that allow for viral persistence are not well understood.

Cytotoxic T lymphocytes (CTL) are thought to be the major host defense responsible for viral clearance from intracellular sites [Oldstone, 1994]. Virus-specific CTL recognize viral antigens in the form of naturally processed peptides, 8–10 residues in length, which are bound in the cleft of HLA class I molecules on the surface of antigen presenting cells [Taylor and Askonas, 1986; Townsend and Bodmer, 1989]. Following recognition of antigen in the context of HLA class I molecules, cell lysis occurs. Although initially beneficial, CTL, in an attempt to limit viral replication, may also mediate tissue damage. Characterization of the CTL response to HCV is thus important in defining the potential dual roles of these cells in disease pathogenesis.

The existence of CTL responses to HCV in chronically infected humans has been reported by several investigators. In chronically infected individuals, multiple proteins serve as targets of the CTL response, yet virus persists [Koziel et al., 1995; Rehermann et al., 1996a]. HLA class I restricted CTLs specific for HCV proteins have been identified among liver infiltrating lymphocytes [Koziel et al., 1992] and in peripheral

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blood [Battegay et al., 1995; Koziel et al., 1995] of patients with chronic HCV infection. In addition, a number of epitopes recognized by HCV-specific CTL have been identified in the context of several different HLA class I molecules [Kita et al., 1993; Shirai et al., 1995; Wentworth et al., 1996], including three HLA-A*0201 restricted CTL epitopes on HCV core protein. Studies of the CTL response have failed to show a clear correlation between HCV-specific CTL responses and clearance of infection. However, it has been suggested that a positive CTL response may be associated with lower titers of the virus [Rehermann et al., 1996b; Hiroishi et al., 1997; Nelson et al., 1997]. Previous studies have demonstrated that dendritic cells are potent antigen presenting cells and are capable of initiating primary in vitro CTL responses [Steinman, 1991]. We describe the use of human peripheral blood dendritic cells to prime $CD8^+$ T cells to HCV core protein, leading to the generation of antigen-specific CTL from uninfected donors. CTL lines from HCV infected patients have been generated in an identical way allowing a comparison of CTL responses between uninfected and infected individuals. We have thus analyzed CTL generated against peptides produced naturally following intracellular processing of viral protein. We obtained dendritic cells by maturation from progenitors under stimulation with granulocyte-macrophage colony stimulating factor (GM-CSF), interleukin-4 (IL-4), and IL-1β [Mehta-Damani et al., 1994]. CTL lines have been generated from HLA-A*0201 positive HCV uninfected and infected donors by culturing $CD8^+$ T cells with autologous dendritic cells loaded with recombinant HCV core protein. The ability of HLA class I restricted HCV core-specific CTL lines generated from uninfected and infected individuals to lyse antigen loaded target cells has been compared.

MATERIALS AND METHODS

Patient Population

All patients and healthy donors used in this study were HLA-A*0201 positive. HCV seronegative healthy donors were drawn from the staff of the Medical School, University of Newcastle upon Tyne. Fourteen HCV seropositive donors (8 female) were available through the Liver Unit of Freeman Hospital, Newcastle upon Tyne. Ten patients were repeatedly HCV RNA positive (Roche Amplicor, Nutley, NJ) and had biopsy proven chronic liver disease, scored as described previously [Ishak et al., 1995], ranging from hepatitis ($n = 8$) to cirrhosis with ($n = 1$) or without ($n = 1$) hepatocellular carcinoma. All HCV RNA positive patients who received alpha-interferon treatment (see Table II) were studied at least 6 months after completion of therapy. The remaining 4 patients were repeatedly HCV RNA negative and had normal liver function tests (including serum alanine transaminase and gamma glutamyl transferase levels). Samples of peripheral blood (50 ml) were collected from each by venipuncture and taken into heparinized tubes with a final concentration of preservative-free calcium heparin of 20 units/ml. The study was approved by the Joint Ethics Committee of the Newcastle and North Tyneside Health Authorities and informed consent was obtained in accordance with guidelines drawn up by this committee.

Peripheral Blood Mononuclear Cells (PBMC)

Human PBMC suspensions were prepared from adult peripheral blood upon Ficoll gradients (Nycomed, Oslo, Norway). PBMCs were washed and finally suspended in RPMI 1640 medium containing 25 mM Hepes buffer and 2 mM L-glutamine (Gibco, Grand Island, NY) and supplemented with 10% v/v heat inactivated autologous plasma (10% RPMI). Approximately 2.5×10^6 PBMCs were centrifuged, resuspended in cryopreservation medium (RPMI 1640 supplemented with 10% v/v DMSO and 20% v/v heat inactivated fetal calf serum), and stored in liquid nitrogen until required. The remainder of the mononuclear cells was then separated into T-cell-enriched (ER+) and T-cell-depleted (ER−) fractions, as previously described [Bhardwaj et al., 1993]. The ER+ fraction was frozen down in cryopreservation medium until required. The ER− fraction was used immediately to obtain a population of cells enriched for dendritic cells.

Dendritic Cell-Enriched Population

Monocytes were depleted from the ER− fraction by panning on dishes coated with human gamma globulin (Sigma, St. Louis, MO), as previously described [Young and Steinman, 1988]. Non-adherent cells were depleted of B cells and NK cells by coating with anti-CD19 and anti-CD56 antibodies (Becton Dickinson, San Jose, CA), respectively, followed by panning on dishes coated with goat anti-mouse IgG (Sigma) [Young and Steinman, 1990]. The resultant population of cells, containing dendritic cell precursors, was resuspended in RPMI supplemented with 1% autologous heat inactivated plasma and incubated at 37°C in the presence of 1,000 units/ml GM-CSF (Peprotech, Rocky Hill, NJ) 5 units/ml IL-4 (Peprotech), and 2.5 units/ml IL-1β (Peprotech) [Mehta-Damani et al., 1994] for 6 days, with addition of cytokines every other day.

T Cells

ER+ cells were thawed rapidly and washed in RPMI. Following the final wash, ER+ cells were depleted of monocytes by panning on gamma globulin-coated dishes. B cells and NK cells were depleted by panning, as above. The resultant T-cell population was enriched for CD8+ T cells by incubation with anti-CD4 (Becton Dickinson), followed by panning, as above.

HCV Core Protein

The portion of the HCV genome encoding the core protein from a genotype 1b isolate was amplified by polymerase chain reaction (PCR) [Milton et al., 1995] and ligated into plasmid pET15b (Novagen, Madison, WI). The plasmid was expressed in *Escherichia coli* strain BL21(DE3)pLysS, to produce core protein with an N-terminal poly-histidine tag. The identity of the

expressed protein was confirmed by Western blotting against anti-core monoclonal antibody (Biogenesis, Poole, UK). The His-tagged protein was solubilized in 6 M urea prior to purification by nickel-affinity chromatography and then refolded on the column (Novagen) by washing with decreasing concentrations of urea. The core protein was finally eluted from the column in 20 mM imidazole buffer and dialyzed against Tris-buffered saline overnight. The concentration of the purified protein was determined using the Biorad (Richmond, CA) Protein Assay kit. A His-tagged protein from human cartilage aggrecan was used as a control for cytotoxicity studies.

Synthetic Peptides

All peptides were synthesized on an Applied Biosystems (Foster City, CA) 431A synthesizer in the Molecular Biology Unit, University of Newcastle upon Tyne. Selection of HCV-derived peptides was based on their predicted binding to HLA-A*0201 molecules [Parker et al., 1994].

Generation of CTL Lines

Cultured dendritic cells were washed and 5 μg/ml of antigen was introduced into the cytoplasm of dendritic cells by osmotic lysis of pinosomes [Okada and Rechsteiner, 1982]. Briefly, cells were incubated with antigen in a hypertonic medium, containing 0.5 M sucrose (BDH) and 10% polyethylene glycol 1500 (BDH) for 20 min at 37°C. Upon return to normal osmotic strength medium, pinocytic vesicles lysed, releasing antigen into the cytoplasm of cells and thus allowing antigen to enter the HLA class I processing pathway. Antigen loaded dendritic cells (1×10^5) were incubated with $CD8^+$ T cells (1×10^6) for 7 days in the presence of 1,000 units/ml GM-CSF, 5 units/ml IL-4, and 2.5 units/ml IL-1β. After 7 days, CTL lines were restimulated with irradiated (30 Gy), autologous PBMC loaded, by osmotic shock, with 5 μg/ml HCV core protein, with the addition of 5 units/ml IL-2. Subsequently, lines were restimulated every 7–10 days.

CTL Assay

A standard 4 hr CTL assay was carried out to assess the ability of CTL lines to lyse autologous target cells. Target cells were washed and either loaded as above, by osmotic shock, with 5 μg/ml full-length core protein or incubated for 1 hr at 37°C with 5 μg/ml core-derived peptides. Antigen loaded autologous adherent mononuclear cells were then incubated with washed effector cells at effector:target ratios of 20:1, 10:1, or 5:1. In antibody blocking experiments, anti-HLA class I (W6/32; IgG2a; Serotech, Bicester, UK) and anti-HLA DR (YE2/36-HLK; IgG2a; Serotech) antibodies were also added to cultures at 20 μg/ml. The amount of cell lysis was assessed by measuring lactate dehydrogenase release in a colorimetric assay (Promega, Madison, WI). For the final 45 min of the incubation, lysis solution was added to the target cell maximum wells to allow the percentage of cytotoxicity to be calculated. Upon addition of the enzyme substrate, the reaction was left to develop before stopping the reaction with H_2SO_4. Plates were read at OD_{492}.

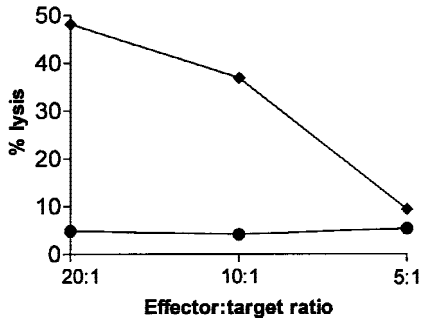

Fig. 1. Cytotoxic activity of CTL line specific for HCV core protein. A representative example of CTL lines obtained from a HCV uninfected healthy donor is shown. The graph demonstrates the % lysis of core protein loaded autologous target cells (♦) and of unpulsed target cells (●) by core protein-specific CTL at 3 effector:target ratios.

The percentage specific lysis was calculated as 100 × [(experimental release – spontaneous release)/(maximum release – spontaneous release)]. Significant cytotoxicity was defined as >10% specific lysis.

RESULTS

Generation of Cytotoxic Effectors by In Vitro Stimulation With HCV Core Protein

Dendritic cells isolated from the peripheral blood of healthy individuals were tested for their ability to present recombinant HCV core protein to autologous, presumably naive, $CD8^+$ T cells. Restimulation of cells every 7–10 days with autologous irradiated PBMC loaded with recombinant HCV core protein and IL-2 (5 units/ml) was required to expand T-cell cultures. No lysis was detected after 4 in vitro stimulations with antigen (data not shown). After 5–6 antigenic stimulations, $CD8^+$ T-cell lines lysed efficiently autologous target cells loaded with HCV core protein but did not lyse untreated target cells (Fig. 1).

HCV Core-Specific CTLs Are Restricted by HLA Class I Molecules

Blocking experiments with an anti-HLA class I antibody demonstrated that the CTL response was HLA class I restricted, as lysis was nearly abrogated in the presence of anti-HLA class I but was almost identical when an isotype-matched control (anti-HLA DR) antibody was coincubated during the CTL assay (Fig. 2). HLA-A*0201 restriction was inferred in HLA mismatch experiments using target cells lacking HLA-A*0201 where no lysis above background was observed (data not shown).

HCV-Specific CTLs From Healthy Donors Recognize Epitopes on Core Protein

HCV core-derived peptides were synthesized based on their predicted binding to HLA-A*0201 molecules and are listed in Table I. Autologous target cells were pulsed with peptide and HCV core-specific CTLs were

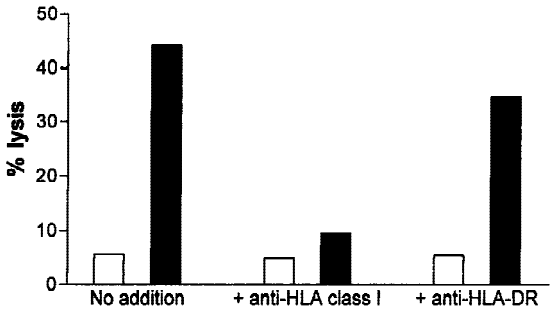

Fig. 2. Inhibition of CTL activity by HLA class I antibodies. HCV core protein-stimulated CTL line was assayed for cytotoxicity to autologous target cells at an effector:target ratio of 20:1 in the presence and absence of anti-HLA class I and control (anti-HLA-DR) antibodies. Open bars: targets without antigen; filled bars: targets loaded with HCV core protein.

tested for their ability to recognize the peptides presented by HLA-A*0201 molecules. All 5 HCV seronegative healthy donors demonstrated a similar pattern of cytotoxicity against the core-derived peptides, and the percentage specific lysis by core protein-specific CTL is shown in Figure 3. Each of the 5 HLA-A*0201 binding peptides were recognized by core-specific CTL. In each case, peptides 177–185 and 35–44 were recognized to the greatest extent. These 2 peptides were predicted to have greater half-times of dissociation from HLA-A*0201 molecules than the remaining 3 peptides (Table I). Peptides 90–98, 132–140, and 156–165 were also recognized by core-specific CTL, but to a lesser extent. The control peptide from influenza matrix protein did not induce CTL activity above background.

Induction of HLA Class I Restricted CTLs in HCV Infected Patients

CTL lines were generated from chronically infected individuals in an identical way to that described for uninfected individuals except that only 2 in vitro stimulations with antigen were required to elicit CTL lines capable of lysing autologous antigen loaded target cells. Table II demonstrates the pattern of peptide recognition by core protein-specific CTL from the HLA-A*0201 positive patients with a history of HCV infection. All 5 peptides were recognized by CTL from the patients, but to differing extents. The CTL responses of the 2 cirrhotic patients, P1 and P2, were poor. The level of CTL responses from the patients varied although, in general, a response to core protein coincided with a response to the 5 core-derived peptides. Only 1 patient, P4, failed to respond to core protein.

The pattern of peptide recognition by HCV infected patients also differed from that of the uninfected healthy donors, with peptides 177–185 and 35–44 no longer being recognized to the greatest extent, and a more even spread of responses to the predicted epitopes observed. The reproducibility of the CTL assay was assessed by obtaining repeat samples from 3 of the patients (Fig. 4). Samples from P2, P12, and P13 were obtained 6, 3, and 6 months apart, respectively. The

TABLE I. Predicted CTL Epitopes Within HCV Core Protein

Position	Sequence	Calculated $T_{0.5}$ dissociation (sec)
35–44	YLLPRRGPRL	363.6
90–98	GLGWVGWLL	160.2
132–140	DLMGYIPLV	44.4
156–165	RVLEDGVNYA	83.4
177–185	FLLALLSCL	836.2

pattern of CTL response from patient P12 varied little over this time, while the repeat sample from P13 still recognized all 5 peptides, although the CTL response to peptides 35–44 and 132–140 had decreased relative to other epitopes. The poor response from cirrhotic pa-

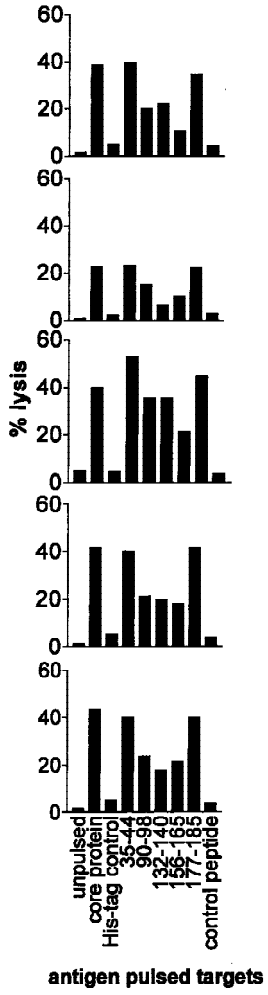

Fig. 3. Lysis of HCV peptide pulsed target cells by core protein-specific CTL from HCV uninfected donors. CTL lines from 5 HCV uninfected donors were generated by in vitro stimulation with core protein. Six antigenic stimulations were required to elicit CTL responses at an effector:target ratio of 20:1 in donors C1 and C2, whereas 5 antigenic stimulations were necessary to elicit CTL responses in the remaining donors. CTL lines from all donors recognize core-derived peptides, with peptides 35–44 and 177–185 being recognized to the greatest extent. Less than 10% lysis was detected when target cells were pulsed with an irrelevant His-tagged protein from human cartilage aggrecan, a control peptide (influenza matrix peptide 58–66), or when unpulsed cells were present.

TABLE II. CTL Activity Against HCV Core Protein and Core-Derived Peptides*

Patient	HCV RNA	Biopsy result	Treatment	Antigen added: Core protein	Core 35–44	Core 90–98	Core 132–140	Core 156–165	Core 177–185	Control peptide	No peptide
P1	+	Cirrhosis	α-IFN non-responder	18.2	18.1	24.0	21.2	19.9	17.9	2.0	0.5
P2	+	Cirrhosis	None	12.1	14.2	16.9	13.3	13.8	13.0	1.0	1.3
P3	–	Not done	None	22.5	18.6	31.2	38.2	43.1	19.6	4.5	4.3
P4	+	Chronic hepatitis	α-IFN transient response	2.6	3.1	0.2	7.5	0.5	3.4	1.0	5.9
P5	–	Not done	None	18.7	31.2	13.4	18.3	29.5	18.6	1.0	3.4
P6	–	Not done	None	59.2	68.7	66.6	75.6	77.9	79.0	4.8	6.6
P7	–	Not done	None	65.2	80.4	69.6	81.6	70.8	85.4	2.0	6.9
P8	+	Minimal hepatitis	None	81.9	47.7	90.2	78.3	44.3	86.9	7.0	7.9
P9	+	Chronic hepatitis	α-IFN non-responder	54.6	63.6	80.8	75.6	73.7	47.3	3.8	7.8
P10	+	Chronic hepatitis	None	69.2	74.8	61.6	65.2	60.1	75.4	2.7	2.2
P11	+	Chronic hepatitis	α-IFN transient response	89.1	68.8	41.9	73.1	66.8	58.9	9.8	8.3
P12	+	Chronic hepatitis	α-IFN transient response	28.8	34.4	27.0	40.3	29.6	39.6	0.2	1.3
P13	+	Minimal hepatitis	α-IFN[a]	41.2	40.9	38.8	60.0	40.1	40.2	4.3	3.9
P14	+	Chronic hepatitis	α-IFN transient response	23.9	22.1	19.7	22.4	21.7	28.8	5.6	7.8

*Values shown are % cytotoxicity by CTL lines generated from 14 donors with a history of HCV infection. Two rounds of *in vitro* antigenic stimulation were used to elicit CTL responses, measured at an effector:target ratio of 10:1. α-IFN, α-interferon.
[a]Patient received α-IFN treatment for 1 month; treatment was then discontinued because of side effects.

tient P2 seen at the time of the first assay had fallen to <10% lysis by the time of the second assay.

DISCUSSION

In this study, it was demonstrated that dendritic cells can be used to generate efficient CTL responses against HCV core protein from uninfected individuals. Previous studies have demonstrated that dendritic cells are potent stimulators of quiescent T cells [Markowicz and Engleman, 1990; Steinman, 1991] and have been shown to induce specific CTLs for a number of viruses, including influenza virus [Bhardwaj et al., 1994] and human immunodeficiency virus [Mehta-Damani et al., 1994]. However, study of CTL responses to HCV has been hampered by the inability to derive infective stocks of the virus. Direct introduction of protein antigens into the cytoplasm of dendritic cells allows the effective presentation by HLA class I molecules without the problems associated with the use of virus vectors, such as vaccinia, as a method of introducing recombinant antigens [Bennink and Yewdell, 1990]. The use of this methodology has allowed us to determine the potential repertoire of CTL responses to HCV proteins and to compare CTL responses of uninfected donors with those of chronically infected HCV patients.

Studies on patients infected chronically with HCV have identified multiple sites within both structural and non-structural proteins of the virus which are targets for HCV-specific CTL [Koziel et al., 1995; Rehermann et al., 1996a]. The use of peptides that represent potential epitopes to stimulate PBMC responses has been used to define CTL epitopes in patients with chronic HCV infection. CTL responses to HCV encoded peptides containing the HLA-A*0201 binding motif have been detected in the peripheral blood of patients following in vitro stimulation with synthetic peptides, including 3 epitopes derived from HCV core protein (35–44, 131–140, 177–185) [Cerny et al., 1995]. Further studies have also reported CTL responses to HCV-derived peptides from HCV seronegative individuals [Cerny and Chisari, 1994; Cerny et al., 1995; Koziel et al., 1997]. Generation of primary responses required multiple rounds of in vitro antigenic stimulation, although estimates of the precursor frequency of the response in seronegative vs. serpositive donors revealed 10–100-fold differences in CTL precursor frequencies [Cerny and Chisari, 1994; Cerny et al., 1995] when quantified by limiting dilution analysis (LDA).

The unique approach to our study has been to generate CTL lines to full-length core protein and to study CTL responses to naturally processed viral protein. Using this approach, HCV core protein-specific CTL lines were generated from HCV uninfected and infected individuals and their ability to lyse antigen loaded and peptide pulsed target cells was assessed. Five distinct core protein epitopes have been identified, 3 of which (35–44, 132–140, and 177–185) have been described previously. We also report the identification of 2 core protein-derived epitopes (90–98 and 156–165) which have not previously been defined. Our identification of 2 new epitopes within the viral core protein, added to the 3 already known, emphasizes the spread of the CTL response to HCV across a large number of epitopes.

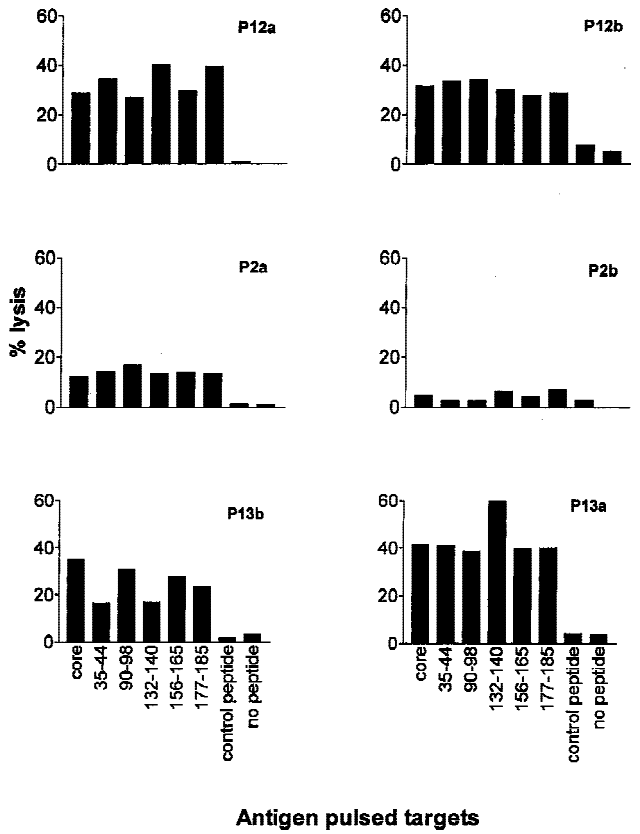

Fig. 4. Lysis of HCV peptide pulsed target cells by core protein-specific CTL from HCV infected donors. CTL lines from 3 patients were generated by in vitro stimulation with core protein on 2 separate occasions. Two antigenic stimulations were required to elicit CTL responses at an effector:target ratio of 10:1. Samples P2b and P13b were obtained 6 months after the first samples and the repeat sample from P12 was obtained after 3 months. The poor response of cirrhotic patient P2 at the time of the first assay had fallen to <10% lysis by the time of the second assay. CTL responses by patient P12 varied little after 3 months, while that of P13 showed a relative decline in response to peptides 35–44 and 132–140. Less than 10% lysis was detected when target cells were pulsed with a control peptide (influenza matrix peptide 58–66), or when unpulsed cells were present.

This contrasts with responses against the restricted number of epitopes that are characteristic of many viral infections [Deng et al., 1997]. One explanation might be that our procedure revealed low frequency, or even normally cryptic, epitopes as a consequence of the strong immunostimulatory properties of dendritic cells loaded at high levels with a single viral protein. Although this explanation might apply to the primary responses from the HCV uninfected donors, the rapid kinetics of the responses from the HCV infected group are consistent with these being secondary responses following in vivo priming.

CTL lines from HCV infected individuals were generated in an identical way to those of uninfected donors, although fewer rounds of antigenic stimulation were required for the generation of CTL capable of eliciting strong cytolytic responses against core peptides. This may reflect differences in CTL precursor (CTL*p*) frequencies between HCV uninfected and infected donors, although this was not formally proven in this study. Work is ongoing to quantitate anti-HCV CTL present in PBMCs from our patient group, using direct flow cytometric quantitation of antigen-specific $CD8^+$ T cells by detecting the binding of peptide loaded class I HLA tetramers to T-cell receptors specific for the individual peptide/class I HLA combination [Altman et al., 1996] and enumeration of interferon-γ (IFN-γ) secreting cells using an ELISPOT assay [Lalvani et al., 1997]. These more direct methods of studying HCV reactive $CD8^+$ cells measure antigen-specific populations directly ex vivo and have advantages over both LDA and conventional cytotoxicity assays of detecting antigen-specific cells rapidly without the bias imposed by in vitro expansion. Previous studies have shown that CTL responses measured in cytotoxic assays do not necessarily correlate with frequency of CTL*p,* as measured by LDA [Rehermann et al., 1996b]. Although the HLA tetramer assay does not measure function, it can be used to obtain important information on cell surface marker phenotypes. The IFN-γ assay is a functional assay and might be expected to correlate with protective CTL responses in vivo.

Thirteen of the patients studied in this report recognized all 5 core-derived peptides, although the pattern of responses differed from that of HCV uninfected donors, with a more even CTL response to each of the peptides. The extent of the CTL response varied quantitatively between the patients included in the study. Although both cirrhotic patients P1 and P2 had CTL responses at the low end of the range, the significance of this observation remains to be established. Overall, no correlation was seen between the presence or strength of CTL response in PBMC and serum HCV RNA status, clinical status, or responsiveness to α-interferon therapy. This is reminiscent of the observation that the frequency of T-helper cell responses in PBMC against HCV core did not differ between untreated patients and those showing either no, or only transient, response to prior α-interferon therapy [Lasarte et al., 1998]. These researchers noted a significantly elevated frequency in patients with a sustained response to α-interferon, a group not studied here. By contrast, the presence, prior to treatment, of intrahepatic CTL activity against peptides derived from HCV core protein has been shown recently to correlate with a subsequent response to α-interferon therapy, either transient or sustained, while most non-responders had undetectable activity [Nelson et al., 1998]. Whether the difference between these findings and those reported above reflects the source of lymphocytes, liver vs. PBMC, or methodology used remains to be established. The finding that 5 of 5 epitopes tested by this procedure are recognized by patient CTLs suggests that the responses to HCV core protein may be more complex than previous studies have indicated. In addition to further epitopes on core protein which remain to be tested, it would be of interest to study other viral proteins with this protocol.

Whatever the role of HCV-specific CTL in controlling viral replication, viremia persists despite the presence

of a cellular immune response in chronically infected individuals. There are a number of general mechanisms by which viruses can escape elimination by the host immune system. Viruses that persist have often evolved strategies to interfere with the pathway that presents viral peptide bound to HLA class I molecules so they can evade attack by CTLs [Hill and Ploegh, 1995]. Acute infection can result in clonal exhaustion where initial CTLs disappear despite persistence of virus [Zinkernagel et al., 1993]. In the case of immunodominant epitopes, viruses may also escape from CTL control by mutation to an altered sequence that cannot be recognized or that acts as an antagonist of the original peptide, rendering CTL unable to lyse infected cells [Pircher et al., 1990; Phillips et al., 1991]. A clear correlation between HCV-specific CTL and clearance of infection has still to be demonstrated, although it has been suggested that a positive CTL response may be associated with lower titers of virus [Rehermann et al., 1996b; Hiroishi et al., 1997; Nelson et al., 1997]. This study has demonstrated that the potential repertoire of CTL responses from uninfected donors is similar to the actual repertoire of chronically infected patients, in that infected individuals can mount CTL responses against the same array of peptides as uninfected individuals. Understanding the mechanisms that allow HCV infection to coexist with a CTL response directed against a wide spectrum of core protein epitopes remains a challenge for future work with important implications for vaccine development.

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